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<b>(21) International Application Number:</b> PCT/US99/11348 <b>(22) International Filing Date:</b> 21 May 1999 (21.05.99)  <b>(30) Priority Data:</b> 60/087,642      2 June 1998 (02.06.98)      US  <b>(71) Applicant:</b> BRISTOL-MYERS SQUIBB COMPANY [US/US]; P.O. Box 4000, Princeton, NJ 08543-4000 (US).  <b>(72) Inventors:</b> VRUDHULA, Vivekananda, M.; 90 Blueberry Hill Reserve, Killingworth, CT 06419 (US). DUBOWCHIK, Gene, M.; 65 Spring Street, Middlefield, CT 06455 (US). DASGUPTA, Bireswar; 215 Ridgefield Drive, Middletown, CT 06457 (US). VYAS, Dolatrai, M.; 19 Thames Way, Madison, CT 06443 (US).  <b>(74) Agent:</b> DUBOFF, Samuel, J.; Bristol-Myers Squibb Company, 5 Research Parkway, Wallingford, CT 06492 (US).			<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> NEUROTROPHIC DIFLUOROAMIDE AGENTS			
<b>(57) Abstract</b>  The present invention relates to the design, synthesis, and the peptidyl-prolyl isomerase (PPIase or rotamase) inhibitory activity of novel $\alpha,\alpha$ -difluoroacetamido compounds that are neurotrophic agents (i.e. compounds capable of stimulating growth or proliferation of nervous tissue) and that bind to immunophilins such as FKBP12 and inhibit their rotamase activity. This invention also relates to pharmaceutical compositions comprising these compounds.			

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## NEUROTROPHIC DIFLUOROAMIDE AGENTS

### BACKGROUND OF THE INVENTION

Immunophilins are cytosolic proteins endowed with peptidyl-  
5 prolyl-*cis-trans* isomerase (PPIase or rotamase) activity. This family of  
proteins behave as chaperone molecules causing *cis-trans* isomerization  
of specific prolyl amide bonds that could be a rate limiting step in the  
correct folding of certain proteins. They are also involved in many  
cellular signal transduction pathways as partners in multiprotein  
10 complexes for which binding in the rotamase active site, but not rotamase  
activity *per se*, appears to be important (Rühlmann, et al., *Immunobiol.*,  
198, pp. 192-206 (1998)). Immunosuppressive drugs such as FK506,  
rapamycin and cyclosporin A bind to specific groups of immunophilins.  
FK506 and rapamycin bind to the so-called FK506-binding proteins (e.g.  
15 FKBP-12, -25, -52), whereas the cyclophilins bind to cyclosporin A. It has  
been shown that binding to the 12kD immunophilin FKBP12 is necessary  
for FK506 to elicit its immunosuppressive activity. Subsequently, it was  
also found that FK506 has two binding domains: one that binds to FKBP12  
and the other (the effector domain) for the complex of FK506 and FKBP12  
20 that binds to the serine/threonine phosphatase, calcineurin. This  
complexation inhibits calcineurin and prevents the proliferation of T-  
lymphocytes, causing immunosuppression. Rapamycin has an effector  
domain of a different structure, and its complex with FKBP12 binds to a  
different target protein that, however, has the same effect of inhibiting T-  
25 lymphocyte proliferation. For a review, see S.L. Schreiber, et al.,  
*Tetrahedron*, 48, pp. 2545-2558 (1992).

While FK506 exhibits immunosuppressive effects, analogs lacking the calcineurin binding effector domain are devoid of immunosuppressive activity. Many small molecules that contain the essential elements of the FKBP12 binding domain of FK506 but lack the calcineurin binding domain were found to retain high affinity binding to FKBP12, and behave as rotamase inhibitors (D.S. Yamshita, et al., *Bioorg. Med. Chem. Lett.*, 4, pp. 325-328 (1994); D.M. Armistead, et al., *Acta Cryst. D*, 51, pp. 522-528 (1995)).

FK506 has been shown to possess neurotrophic properties *in vitro* and *in vivo* (W.E. Lyons, et al., *Proc. Natl. Acad. Sci USA*, 91, pp. 3191-3195 (1994); B.G. Gold, et al., *J. Neurosci.*, 15, pp. 7509-7516 (1995)). However, its immunosuppressive properties as well as other serious side effects are drawbacks to its use as a neuroregenerative agent. Recently, *in vitro* studies in PC12 cells, SY5Y cells, and chick sensory dorsal root ganglion explant cultures have shown that small molecule, nonimmunosuppressive FKBP12 rotamase inhibitors also promote neurite outgrowth, and a number of these compounds have shown utility in reversal of CNS lesioning and nerve crush in animal models (G.S. Hamilton, et al., *Curr. Pharm. Design*, 3, pp. 405-428 (1997); B.G. Gold, et al., *Exp. Neurol.*, 147, pp. 269-278 (1997)). Thus, while the calcineurin binding domain of FK506 is necessary for immunosuppressive activity, it is not required for neurotrophic activity.

A 10-50 fold elevated expression of immunophilins in the central nervous system in comparison with the immune system is well documented (S.H. Snyder, et al., *Nature Med.*, 1, pp. 32-37 (1995)). Recently, augmented expression of FKBP12 m-RNA following facial

nerve crush and sciatic nerve lesions was established in facial and lumbar motor neurons. The observed augmentation paralleled the enhanced expression of growth associated protein GAP43 mRNA (B.G. Gold, et al., *Neurosci. Lett.*, 241, pp. 25-28 (1998)). These observations make FKBP12 an attractive target for developing nonimmunosuppressive rotamase inhibitors which promote neurite outgrowth. Such compounds are potential therapeutics to reverse neuronal damage caused by neurodegenerative disease or physical trauma.

10        There have been disclosures of related compounds for overcoming multidrug resistance (MDR) or as immunosuppressants such as:

WO 94/07858 published 4/14/94

WO 92/19593 published 11/12/92

U.S. Patent 5,622,970 granted 4/22/97

15        U.S. Patent 5,330,993 granted 7/19/94

U.S. Patent 5,192,773 granted 3/9/93

U.S. Patent 5,516,797 granted 5/14/96

WO 92/21313 published 12/10/92

European Application 564924 published 10/13/93

20        European Application 405994 published 1/2/91

Other prior art disclosing related compounds having neurotrophic activity are:

25        WO 96/40140 published 12/19/96

WO 96/40633 published 12/19/96

WO 97/16190 published 5/9/97

WO 96/41609 published 12/27/96

U.S. Patent 5,696,135 granted 12/9/97

WO 97/36869 published 10/9/97

U.S. Patent 5,721,256 granted 2/24/98

U.S. Patent 5,654,332 granted 8/5/97

5 WO 98/13343 published 4/2/98

WO 98/13355 published 4/2/98

Since there are relatively few FKBP12-binding compounds that are known to stimulate neurite growth, there remains a great need for  
10 additional neurotrophic, FKBP12-binding compounds.

### SUMMARY AND OF THE INVENTION

Surprisingly, applicant has solved the aforementioned problem.  
15 The present invention relates to novel  $\alpha,\alpha$ -difluoro substituted acetamide compounds and pharmaceutical compositions thereof that possess neurotrophic properties.

### DETAILED DESCRIPTION OF THE INVENTION

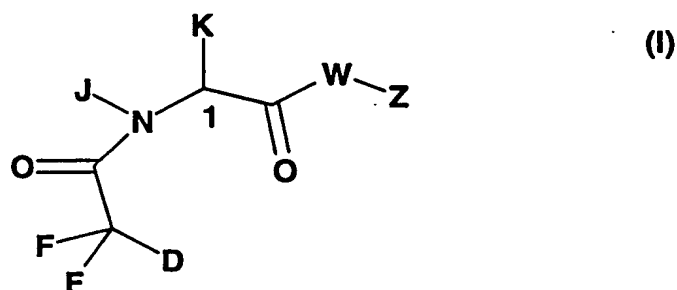
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According to one embodiment, the present invention provides:

A compound with affinity for an FK506 binding protein having the formula (I):

25

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and pharmaceutically acceptable salts thereof:

5 wherein W is CH<sub>2</sub>, O, NH, or N-(C<sub>1</sub>-C<sub>4</sub>)-alkyl;

wherein J is hydrogen, (C<sub>1</sub>-C<sub>4</sub>)-alkyl or benzyl;

10 wherein K is (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, benzyl or cyclohexylmethyl, or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain a heteroatom selected from the group consisting of O, S, SO, and SO<sub>2</sub>;

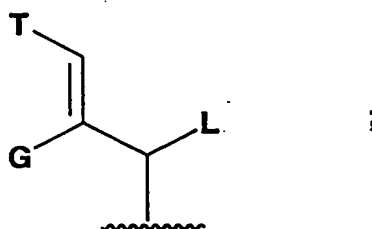
15 wherein the stereochemistry at carbon position 1 is R or S;

wherein Z is Q or -(CH<sub>2</sub>)<sub>m</sub>-C(H)Q'A;

wherein m is 0-3;

20 wherein Q is hydrogen, CHL-Ar, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl, Ar substituted (C<sub>1</sub>-C<sub>6</sub>)-alkyl, (C<sub>2</sub>-C<sub>6</sub>)-alkenyl or

6



wherein L and G are independently hydrogen, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl;

5

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C<sub>1</sub>-C<sub>4</sub>)-alkyl or O-(C<sub>2</sub>-C<sub>4</sub>)-alkenyl and carbonyl;

- 10 wherein D is (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl or (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl substituted with (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>4</sub>)-straight or branched alkenyl, O-(C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, O-(C<sub>2</sub>-C<sub>4</sub>)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C<sub>1</sub>-C<sub>4</sub>)-alkyl or (C<sub>2</sub>-C<sub>4</sub>)-alkenyl]-Ar  
 15 or Ar;

- wherein Ar is a carbocyclic aromatic group selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl, and anthracenyl; or a heterocyclic aromatic group selected from  
 20 the group consisting of 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indoliziny, indolyl, isoindolyl, 3H-indolyl, indoliny, benzo[b]furanyl,  
 25 benzo[b]thiophenyl, 1H-indazolyl, benzimidazolyl, benzthiazolyl, purinyl,



4H-quinoliziny, quinoliny, isoquinoliny, cinnoliny, phthalaziny, quinazoliny, quinoxaliny, 1,8-naphthyridiny, pteridiny, carbazolyl, acridiny, phenaziny, phenothiaziny, and phenoxaziny;

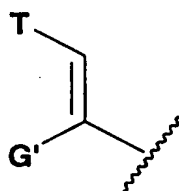
- 5 wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halogen, hydroxyl, hydroxymethyl, nitro, trifluoromethyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl, O-[(C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl], O-benzyl, O-phenyl, 1,2-
- 10 methylenedioxy, amino, carboxyl, N-[(C<sub>1</sub>-C<sub>5</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>5</sub>)-straight or branched alkenyl] carboxamides, N,N-di-[(C<sub>1</sub>-C<sub>5</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>5</sub>)-straight or branched alkenyl] carboxamides, N-morpholinecarboxamide, N-benzylcarboxamide, N-thiomorpholinocarboxamide, N-picolinoylcarboxamide, O-X, CH<sub>2</sub>-(CH<sub>2</sub>)<sub>p</sub>-
- 15 X, O-(CH<sub>2</sub>)<sub>p</sub>-X, (CH<sub>2</sub>)<sub>p</sub>-O-X, and CH=CH-X;

wherein X is 4-methoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrazyl, quinolyl, 3,5-dimethylisoxazoyl, isoxazoyl, 2-methylthiazoyl, thiazoyl, 2-thienyl, 3-thienyl, or pyrimidyl;

- 20 wherein p is 0-2;

- wherein Q' and A are independently hydrogen, Ar, (C<sub>1</sub>-C<sub>10</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>10</sub>)-straight or branched alkenyl or alkynyl, (C<sub>5</sub>-C<sub>7</sub>)cycloalkyl substituted-straight (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl,
- 25 (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl substituted (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl, or Ar substituted (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl wherein, in each case, any

one of the CH<sub>2</sub> groups of said alkyl, alkenyl or alkynyl chains may be optionally replaced by a heteroatom selected from the group consisting of O, S, SO, SO<sub>2</sub> and NR, wherein R is selected from the group consisting of hydrogen, (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>4</sub>)-straight or branched alkenyl or alkynyl, and (C<sub>1</sub>-C<sub>4</sub>)-bridging alkyl wherein a bridge is formed between the nitrogen and a carbon atom of said heteroatom-containing chain to form a ring, and wherein said ring is optionally fused to an Ar group; or



; and

wherein G' is hydrogen, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl.

Another embodiment of this invention are compounds of formula I wherein Z is  $-(CH_2)_m-C(H)Q'A$ .

A preferred embodiment are compounds of formula I wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is 3-phenylpropyl; and A is 3-(3-pyridyl)propyl.

Another preferred embodiment are compounds of formula I wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is 3-phenylpropyl; and A is 3-(3-pyridyl)propyl.

Another preferred embodiment are compounds of formula I wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-  
5 trimethoxyphenyl; Q' is phenyl; and A is 2-phenylethyl.

Another preferred embodiment are compounds of formula I wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-  
10 trimethoxyphenyl; Q' is phenyl; and A is 2-phenylethyl.

Another preferred embodiment are compounds of formula I wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-  
15 trimethoxyphenyl; and Q' and A are both (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyls substituted at the terminal end with a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.

Another preferred embodiment are compounds of formula I  
20 wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; and Q' and A are both (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyls substituted at the terminal end with a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.

25

Another preferred embodiment are compounds of formula I wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-

trimethoxyphenyl; Q' is a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar;  
and A is a (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyl substituted at the terminal end with  
a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.

- 5           Another preferred embodiment are compounds of formula I  
wherein J and K are taken together to form a pyrrolidine ring; the  
stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-  
trimethoxyphenyl; Q' is a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar;  
and A is a (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyl substituted at the terminal end with  
10 a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.

Another aspect of the present invention provides for a  
pharmaceutical composition which comprises as an active ingredient an  
amount of a compound of formula I, or a pharmaceutically acceptable salt  
15 thereof, effective for stimulating neurite growth in nerve cells, and one or  
more pharmaceutically acceptable carriers, excipients or diluents thereof.

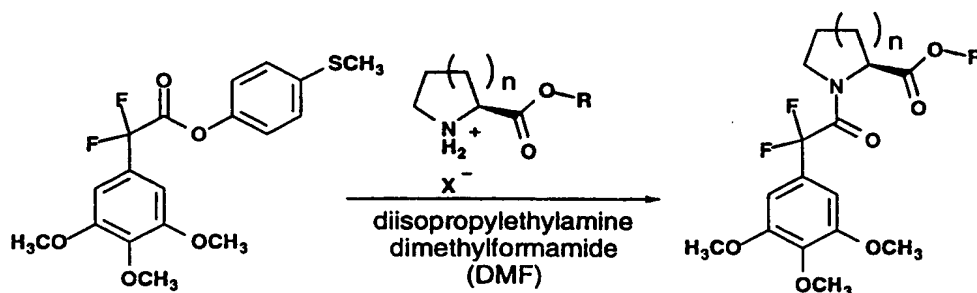
Another aspect of the present invention provides for a method for  
stimulating neurite growth in nerve cells comprising the step of  
20 contacting said nerve cells with a composition comprising a neurotrophic  
amount of a compound of formula I with affinity for an FK-506 binding  
protein.

Another aspect of the present invention provides for a method for  
25 stimulating neurite growth in nerve cells comprising the step of  
contacting said nerve cells with a composition comprising a neurotrophic  
amount of a compound of formula I with affinity for FKBP12.

### GENERAL SUMMARY OF COMPOUND PREPARATION

The syntheses of the examples described in Table 1 was carried out using one of the methods described below that are commonly employed in peptide chemistry (see M. Bodanszky and A. Bodanszky, "The Practice of Peptide Synthesis," Springer-Verlag, Berlin (1984)):

A) An acylation reaction involving *p*-methylthiophenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid with appropriate prolyl or pipercolate ester in dimethylformamide (DMF) in the presence of diisopropylethylamine.

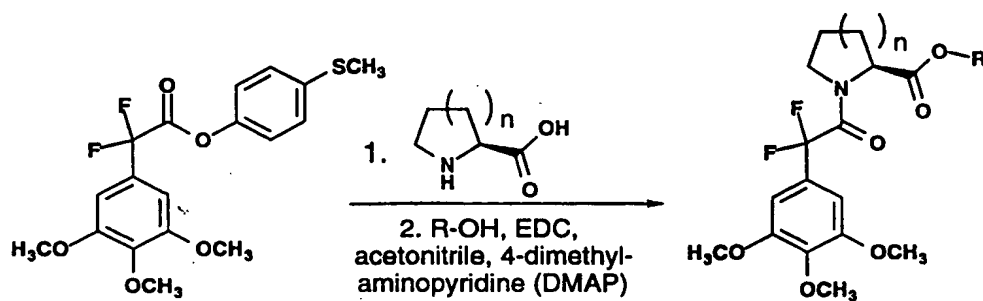


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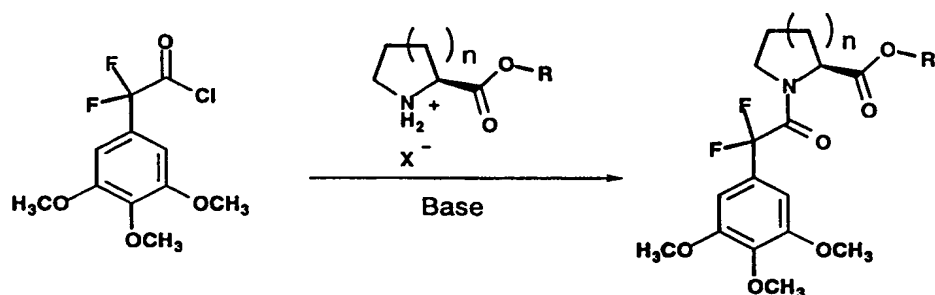
B) Acylation of proline or pipercolic acid with the *p*-methylthiophenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid, followed by esterification of the resulting acid with the appropriate alcohol using a water soluble carbodiimide coupling reagent in acetonitrile.

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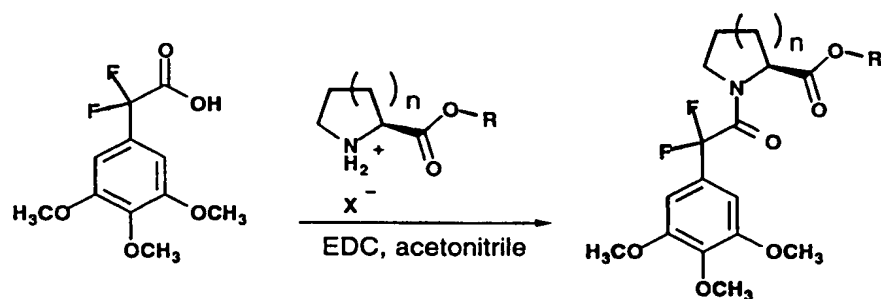
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C) Schotten-Baumann reaction of *in situ* generated  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetyl chloride with the appropriate prolyl or  
 5    pipicolate ester.



D) Peptide coupling using carbodiimide or a mixed anhydride  
 10    approach were also used in some cases.

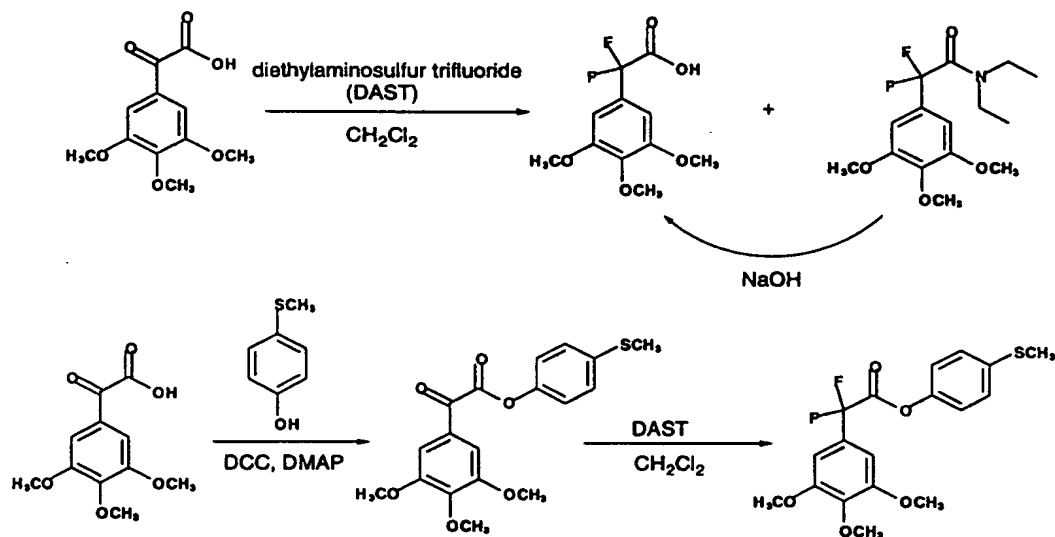


The  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid or its *p*-  
 15    methylthiophenolic ester required for the above three approaches were  
 synthesized by fluorination of the parent keto compound with

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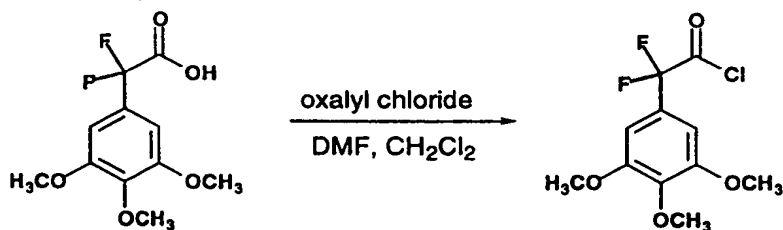
diethylaminosulfurtrifluoride. In the case of the fluorination of 3,4,5-trimethoxyphenyl- $\alpha$ -oxoacetic acid, the corresponding N,N-diethylamide was also obtained. This N,N-diethylamide could be easily converted to the desired acid by alkaline hydrolysis.

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The  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid was converted to the corresponding acid chloride using oxalyl chloride and catalytic dimethylformamide in methylene chloride.

10



15

### Preparation of Reagents

#### General

<sup>1</sup>H NMR spectra in deuterated chloroform were run on a Bruker AC-300 or a Varian Gemini 300 spectrometer and chemical shifts were reported in ppm (δ) with reference to tetramethylsilane. All evaporations were carried out on a rotary evaporator under reduced pressure. Magnesium sulfate was used for drying the organic layer after extractive work up. LC-MS analysis were carried out on a Shimadzu instrument using either of the following two systems: System 1 consists of a PHX-LUNA C18 column (4.6 x 30 mm) employing a 4 min linear gradient of 20% to 100% solvent B:A (solvent A: 10% methanol, 90% water, 0.1% trifluoroacetic acid; solvent B: 90% methanol, 10% water, 0.1% trifluoroacetic acid) with the UV detector set at 220 nm. System 2 consists of a YMC C18 column (4.6 x 50 mm) employing a 4 or 8 min linear gradient of 0% to 100% solvent B:A with other conditions as described above for system 1. The water soluble carbodiimides used were either the hydrochloride salt or the methiodide of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). Abbreviations used were in accordance with the guidelines provided by the American Chemical Society for their publications.

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#### ***p*-(Methylthio)phenolic ester of 3,4,5-trimethoxyphenyl-α-oxoacetic acid**

To a stirred solution of 3,4,5-trimethoxyphenyl-α-oxoacetic acid (12.0 g, 49.9 mmol) in anhydrous acetonitrile (150 mL) at ambient temperature was added 4-(methylthio)-phenol (8.40 g, 59.9 mmol), dicyclohexylcarbodiimide (DCC) (15.4 g, 74.9 mmol) and 4-dimethylaminopyridine (0.428 g, 3.50 mmol) under nitrogen. The reaction mixture was stirred for 8h, then cooled to 0°C in an ice bath and 1M solution of oxalic acid in acetonitrile was added. The precipitated



dicyclohexylurea was removed by filtration. The filtrate was diluted with ethyl acetate (400 mL) and the organic layer was washed with water (3 x 200 mL), brine (200 mL) and dried. The solvent was evaporated to give a pale yellow solid which was recrystallized from 2-propanol to give the pure ester (11.0 g, 61% yield). MS: M+H = 363. <sup>1</sup>H-NMR: 7.41 (s, 2H), 7.35 (d, J = 8.7 Hz, H<sub>2</sub> & H<sub>6</sub>, 2H), 7.21 (d, J = 8.7 Hz, H<sub>3</sub> & H<sub>5</sub>, 2H), 3.99 (s, 3H), 3.94 (s, 6H), 2.52 (s, 3H).

10 *p*-(Methylthio)phenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid.

To a stirred solution of the *p*-(methylthio)phenolic ester of 3,4,5-trimethoxyphenyl- $\alpha$ -oxoacetic acid (1.00 g, 2.76 mmol) in anhydrous methylene chloride (10 mL) at room temperature was added diethylaminosulfurtrifluoride (DAST) (4.44 g, 27.6 mmol) under nitrogen. 15 The reaction mixture was stirred overnight. It was then cooled in an ice bath and excess DAST was quenched by dropwise addition of water. Ethyl acetate (150 mL) was then added and the organic layer was washed repeatedly with water until the pH of the aqueous layer was neutral. The organic layer was then washed with brine (50 mL), dried, and the solvent 20 evaporated to afford the title compound (0.961 g, 2.50 mmol, 91%) as a brown solid. <sup>1</sup>H-NMR: 7.27 (d, J = 8.7 Hz, H<sub>2</sub> & H<sub>6</sub>, 2H), 7.04 (d, J = 8.8 Hz, H<sub>3</sub> & H<sub>5</sub>, 2H), 6.92 (s, 2H), 3.92 (s, 6H), 3.91 (s, 3H), 2.48 (s, 3H).

$\alpha,\alpha$ -Difluoro-3,4,5-trimethoxyphenylacetic acid.

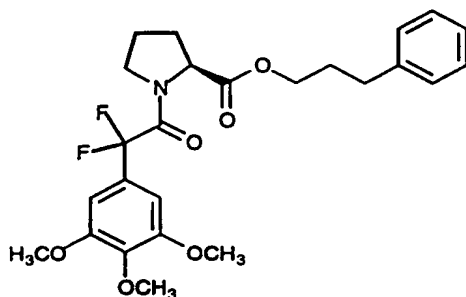
25 To a stirred solution of 3,4,5-trimethoxyphenyl- $\alpha$ -oxoacetic acid (3.81 g, 15.8 mmol) in anhydrous methylene chloride (30 mL) at room temperature was added DAST (20.4 g, 127 mmol) under nitrogen and the mixture was stirred overnight. The mixture was then cooled in an ice

bath and excess DAST was quenched by dropwise addition of water. Ethyl acetate (300 mL) was added and the organic layer was washed with saturated aqueous sodium bicarbonate (2 x 100 mL) followed by water (100 mL). The residue obtained after drying and evaporation was purified by silica gel chromatography, eluting with hexane/ethyl acetate (9:1 to 7:3), to give the N,N-diethylamide derivative (2.10 g, 6.62 mmol, 42%) as a pale yellow solid. <sup>1</sup>H-NMR: 6.77 (s, 2H), 3.88 (s, 9H), 3.45 (q, J = 7.0 Hz, 2H), 3.25 (q, J = 7.0 Hz, 2H), 1.20 (t, J = 7.0 Hz, 3H), 1.10 (t, J = 6.9 Hz, 3H). Bicarbonate washing after acidification and extractive work up with ethylacetate gave the crude title compound. Purification by reversed phase column (C18) chromatography eluting with water/methanol/trifluoroacetic acid (69.9:30:0.1) gave the pure difluoro acid (0.616 g, 2.34 mmol, 15%) as a white solid. <sup>1</sup>H-NMR: 6.85 (s, 2H), 3.90 (s, 6H), 3.89 (s, 3H). Anal. C: 50.59, H: 4.72, F: 14.24 (found), C: 50.39, H: 4.61, F: 14.49 (calcd). The N,N-diethylamide (2.00 g, 6.30 mmol) obtained above was hydrolyzed to the title acid by heating a solution in ethanol (5 mL) with 10% sodium hydroxide (13 mL) at reflux for 4 h. Acidification followed by extractive work up with ethyl acetate gave the crude acid which was purified as described above to give 1.51 g of the title compound as a white solid.

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 **$\alpha,\alpha$ -Difluoro-3,4,5-trimethoxyphenylacetylchloride**

A stirred solution of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid (1.50 g, 6.63 mmol) in anhydrous methylene chloride (20 mL) was treated with oxalyl chloride (2.52 g, 19.8 mmol) and 1 drop of dimethylformamide. After vigorous effervescence ceased, the reaction mixture was stirred for 3h. The solvents were evaporated and traces of oxalyl chloride were removed by repeated evaporation with anhydrous methylene chloride to give the acid chloride (1.61 g, 99% yield) as a dark yellow solid.

**Example 1**

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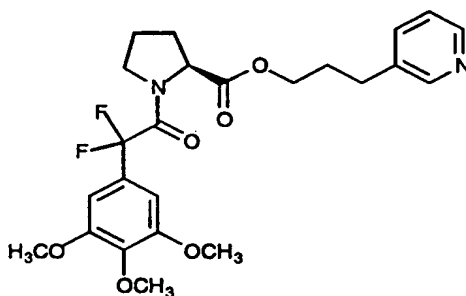
A suspension of N-Boc-L-proline (6.04 g, 28.0 mmol), 3-phenylpropanol (4.58 g, 33.6 mmol), DCC (8.68 g, 42.0 mmol), and 4-dimethylaminopyridine (0.210 g, 1.72 mmol) in anhydrous ether (60 mL) was stirred under nitrogen for 8h. Water (200mL) was added and the mixture was extracted with ethyl acetate (3 x 200 mL). The organic layer was separated and washed with water (2 x 100mL), brine (2 x 100 mL), dried and evaporated. Purification of the residue by silica gel chromatography eluting with hexane/ethyl acetate (7:3 to 7:3) gave the pure phenylpropyl ester (7.62 g, 81%) as a colorless oil. MS: M+H = 334.

<sup>1</sup>H NMR: 7.26 (m, 2H), 7.17 (m, 3H), 4.31 & 4.21 (dd, J = 8.1, 3.2 Hz, 1H), 4.09 (m, 2H), 3.59-3.32 (m, 2H), 2.64 (t, J = 8.9 Hz, 2H), 2.19 (m, 1H), 1.91 (m, 5H), 1.42 (s, 4H), 1.39 (s, 6H). A portion of this material (0.393 g, 1.18 mmol), in methylene chloride (8 mL) was treated with trifluoroacetic acid (0.13 mL). After 1h volatiles were evaporated and a solution of the resulting trifluoroacetate salt in dimethylformamide (12 mL) was combined with *p*-methylthiophenolate ester of 3,4,5-trimethoxy- $\alpha,\alpha$ -difluorophenyl acetic acid (0.428 g, 1.18 mmol) and diisopropylethylamine (0.152 g, 1.18 mmol). The solution was stirred for 18h, and then diluted with ethyl acetate (100 mL), washed with water (50 mL), and dried. The solvent was removed by

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evaporation. The residue was purified by silica gel chromatography, eluting with hexane/ethyl acetate (9:1 to 4:1) to give the product (0.360 g, 0.755 mmol, 64%) as a brown oil. (M+H) = 478. <sup>1</sup>H NMR: 7.25 (m, 2H), 7.17 (m, 3H), 6.87 (s, 2H), 4.57 (m, 1H), 4.10 (m, 2H), 3.87 (s, 6H), 3.85 (s, 3H), 3.57 (m, 2H), 2.68 (t, *J* = 7.5 Hz, 2H), 2.16 (m, 1H), 1.96 (m, 5H).

### Example 2

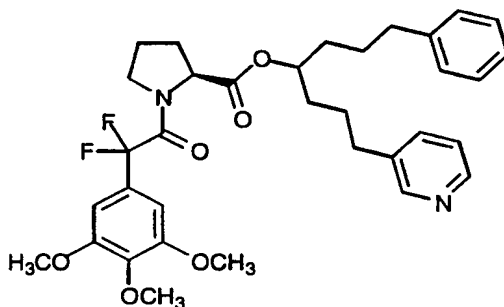


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Esterification of N-Boc-L-proline (1.01 g, 4.60 mmol) with 3-(3'-pyridyl)propan-1-ol (0.772 g, 5.60 mmol) was carried out using DCC (1.45 g, 7.00 mmol) and 4-dimethylaminopyridine (0.102 g, 0.834 mmol) in anhydrous ether (25 mL). Work up was carried out as described above for example 1. Purification by silica gel chromatography, eluting with hexane/ethyl acetate (7:3 to 3:7) gave the N-Boc-prolylester of 3-(3'-pyridyl)propan-1-ol (1.34 g, 4.00 mmol, 87%) as a colorless oil. MS: M+H = 335. <sup>1</sup>H-NMR: 8.42 (m, 2H), 7.50 (m, 1H), 7.20 (m, 1H), 4.36 & 4.22 (dd, *J* = 8.0, 3.0 Hz, 1H, rotamers), 4.17 (m, 2H), 3.49 (m, 2H), 2.70 (t, *J* = 10.0 Hz, 2H), 2.20 (m, 1H), 1.91 (m, 5H), 1.43 (s, 4.5H), 1.39 (s, 4.5H). The N-Boc-prolylester of 3-(3'-pyridyl)propan-1-ol (0.271 g, 0.811 mmol), was deprotected and coupled with the *p*-methylthiophenolate ester of 3,4,5-trimethoxy- $\alpha,\alpha$ -difluorophenyl acetic acid (0.312 g, 0.811 mmol), diisopropylethylamine (0.104 g, 0.811 mmol) in dimethylformamide (20

mL) as described in example 1. The crude product was partitioned between ethyl acetate (200 mL) and 1M hydrochloric acid (2 x 25 mL). The aqueous layer was basified with sodium bicarbonate and extracted with ethyl acetate (3 x 100 mL). The solution was evaporated after washing with water and drying. Purification of the residue by silica gel chromatography, eluting with chloroform/methanol (99.3:0.7) provided the title compound (0.331 g, 0.692 mmol, 85%) as pale brown oil.  $M+H = 479$ .  $^1H$ -NMR: 8.38 (m, 2H), 7.45 (m, 1H), 7.18 (m, 1H), 6.83 (s, 2H), 4.57 & 4.53 (dd,  $J = 8.5, 3.2$  Hz, 1H, rotamers), 4.17 (m, 1H), 4.06 (m, 1H), 3.83 (s, 6H), 3.81 (s, 3H), 3.53 (m, 2H), 2.66 (t,  $J = 7.6$  Hz, 2H), 2.12 (m, 1H), 1.88 (m, 5H).

### Example 3

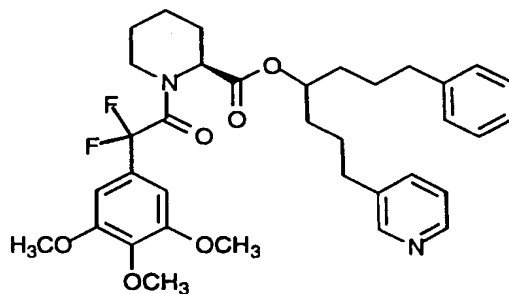


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The coupling of L-proline with the *p*-(methylthio)phenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid was carried out as described above for example 2. The trimethoxydifluorophenylacetamidoproline derivative was obtained in 89% yield after purification by silica gel chromatography, eluting with methylene chloride/ethyl acetate/acetic acid (70:27.5:2.5).  $M+H = 360$ .  $^1H$ -NMR: 8.51 (br, OH), 6.88 (s, 2H), 4.65 (m, 1H), 3.89 (s, 9H), 3.55 (m, 2H), 2.20 (m, 2H), 1.98 (m, 2H). LC-MS: System 1,  $t_R = 5.2$  min. Water soluble carbodiimide (EDC hydrochloride, 0.318 g, 1.66

mmol) mediated esterification of the intermediate acid (0.400 g, 1.11 mmol) with 1-phenyl-7-(3'-pyridyl)-heptan-4-ol (0.358 g, 1.33 mmol), in the presence of a catalytic amount of dimethylaminopyridine (0.101 g, 0.830 mmol) in acetonitrile (25 mL) gave, after extractive work up with ethyl acetate and water, the crude product. Purification by silica gel chromatography gave the title compound (0.425 g, 0.695 mmol, 63%) as a yellow oil. MS: M+H = 611. <sup>1</sup>H-NMR: 8.36 (m, 2H), 7.58 - 7.39 (m, 1H), 7.22 - 7.07 (m, 6H), 6.80 (d, J = 3.1 Hz, 2H), 4.88 (m, 1H), 4.48 (m, 1H), 3.79 (m, 9H), 3.44 (m, 2H), 2.54 (m, 4H), 2.08 (m, 1H), 1.83 (m, 2H), 1.52 (m, 9H). LC-MS: System 2 (8 min gradient), t<sub>R</sub> for the diastereomers was 6.9 and 7.2 min.

#### Example 4



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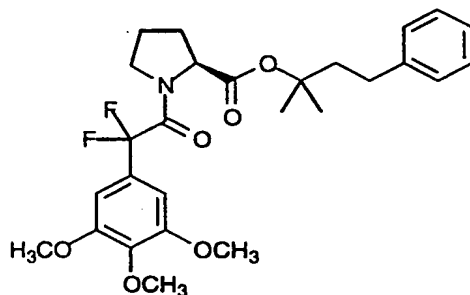
N-Boc-L-pipecolic acid (0.280 g, 1.22 mmol) was esterified with 1-phenyl-7-(3'-pyridyl)-heptan-4-ol (0.394 g, 1.46 mmol) employing EDC hydrochloride (0.350 g, 1.83 mmol) and 4-dimethylaminopyridine (0.081 g, 0.663 mmol) in acetonitrile (25 mL). Purification by silica gel chromatography, eluting with chloroform/methanol (100:0 to 99:1), gave the pure 1-phenyl-7-(3'-pyridyl)-heptan-4-ol ester of N-Boc-pipecolic acid in 85% yield (0.498 g) as a colorless oil. MS: M+H = 481. LC-MS: System 2 (4 min gradient), t<sub>R</sub> = 3.8 min. A portion of this N-Boc ester (0.369 g, 0.768 mmol) was deprotected

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as described in example 1 with trifluoroacetic acid. The crude product was then coupled with  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid (0.241 g, 0.921 mmol), using EDC hydrochloride (0.220 g, 1.15 mmol) and 4-dimethylaminopyridine (0.101 g, 0.826 mmol) in acetonitrile (25 mL).

- 5 After extractive work up as described in example 1, the product was purified by silica gel chromatography, eluting with hexane/ethyl acetate (4:1 to 1:1), to give the title compound (0.624 g, 1.05 mmol, 78%) as a pale yellow oil. MS:  $M+H = 625$ .  $^1H$ -NMR: 8.38 - 8.34 (m, 2H), 7.75 - 7.00 (m, 7H), 6.76 - 6.63 (m & s, 2H), 5.25 - 4.10 (4 x m, 2H), 3.80 - 3.50 (m, 10H), 3.00 - 2.85 (m, 1H), 2.52 (m, 2H), 2.25 - 1.00 (4 x m, 16H). LC-MS: System 2 (4 min gradient),  $t_R$  for the diastereomers = 3.6 and 3.8 min. Anal.  $C_{35}H_{42}N_2O_6F_2$ , C = 67.40, H = 6.85, N = 4.23, F = 5.86 (found), C = 67.29, H = 6.78, N = 4.48, F = 6.08 (calcd.).
- 10

15 **Example 5**



- N-Boc-L-proline (2.01 g, 9.29 mmol) was esterified with 1,1-dimethyl-3-phenyl propanol (1.83 g, 11.1 mmol) using EDC methiodide (4.14 g, 13.9 mmol) and 4-dimethylaminopyridine (0.100 g, 0.815 mmol) in anhydrous acetonitrile (30 mL) as described in example 4. Following aqueous/organic extractive work up, purification by silica gel chromatography, eluting with hexane:ethyl acetate (95:5 to 80:20), gave pure 1,1-dimethyl-3-phenylpropyl
- 20

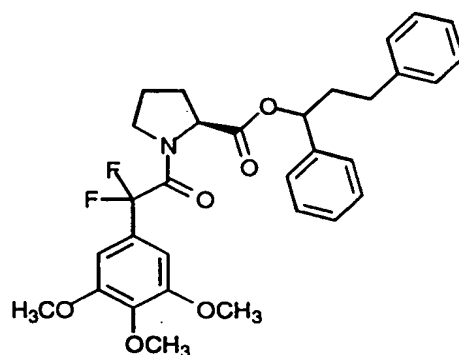
ester (1.13 g, 3.13 mmol, 34%) as a colorless oil. MS:  $M+H = 362$ .  $^1H$ -NMR: 7.29 (m, 2H), 7.18 (m, 3H), 4.23 & 4.17 (dd,  $J = 8.9$  &  $3.4$  Hz, 1H), 3.60-3.35 (m, 2H), 2.65 (m, 2H), 2.27-1.83 (br m, 6H), 1.46 (s, 4H), 1.44 (s, 6H). A portion of this Boc-protected ester (0.689g, 1.90 mmol) was deprotected with

5 trifluoroacetic acid as described in example 1 and acylated with the *p*-(methylthio)phenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid (0.533 g, 1.39 mmol) in the presence of diisopropylethylamine (0.246 g, 1.90 mmol) in dimethylformamide (10 mL). After extractive work up using ethyl acetate, the crude product was purified by silica gel

10 chromatography, eluting with hexane/ethyl acetate (9:1 to 7:3), to give the title compound (0.350 g, 0.693 mmol, 36%) as a pale brown oil. MS:  $M+H = 506$ .  $^1H$ -NMR: 7.27 (m, 2H), 7.16 (m, 3H), 6.90 (s, 2H), 4.52 (m, 1H), 3.86 (s, 9H), 3.57 (m, 2H), 2.75 - 2.50 (m, 2H), 2.20 - 1.87 (m, 6H), 1.49 (d,  $J = 2.7$  Hz, 6H).

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### Example 6



20 The coupling of L-proline with the *p*-(methylthio)phenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid was carried out as described in example 5. The intermediate trimethoxydifluorophenylacetamidoproline

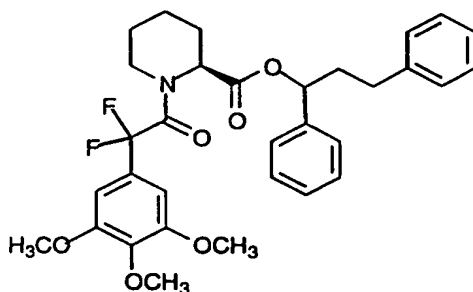


derivative (0.455 g, 1.26 mmol) was esterified with 1,3-diphenylpropanol (0.325 g, 1.51 mmol) using EDC hydrochloride (0.362 g, 1.90 mmol) and 4-dimethylaminopyridine (0.081g, 0.656 mmol) in anhydrous acetonitrile (20 mL). Following extractive work up, purification by silica gel

- 5 chromatography, eluting with hexane/ethyl acetate (9:1 to 7:3), gave the desired compound (0.182 g, 0.328 mmol, 26%) as a colorless oil. MS: M+H = 554. <sup>1</sup>H-NMR: 7.31 (m, 7H), 7.20 (m, 3H), 6.81 (s, 2H), 5.74 (m, 1H), 4.71 (m, 1H), 3.88 (d, J=7.1 Hz, 3H), 3.78 (d, J =5.5 Hz, 6H), 3.59 (m, 2H), 2.63 (m, 2H), 2.33-1.85 (m, 6H). LC-MS: System 2 (8 min gradient), t<sub>R</sub> = 8.2 min.

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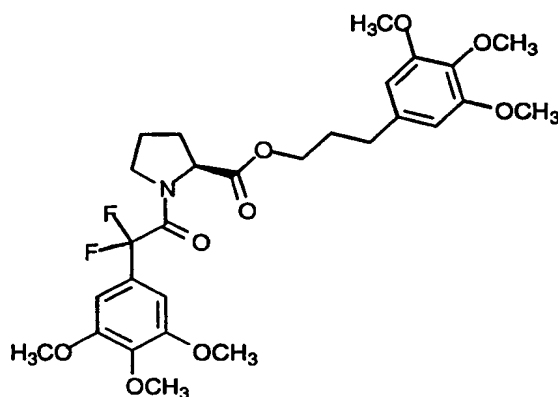
### Example 7



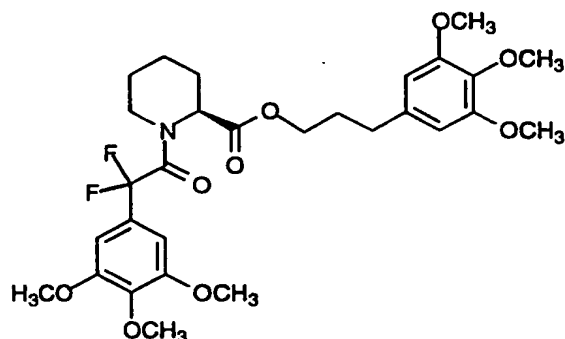
- 15 N-Boc pipecolic acid (0.345 mg, 1.5 mmol) was esterified as described in example 5 with 1,3-diphenyl-1-propanol to obtain the diphenylpropyl ester in 45% yield. MS: M+H = 424. LC-MS: System 2 (4 min gradient), t<sub>R</sub> = 4.8 min. A portion of this N-Boc-protected ester (270 mg, 0.64 mmol) was deprotected with trifluoroacetic acid as described in example 1. The
- 20 resulting trifluoroacetate salt in dichloromethane (4 mL) was acylated with freshly prepared α,α-difluoro-3,4,5-trimethoxyphenylacetyl chloride (201 mg, 1.2 equiv). After 15 h, the solvent was evaporated and the residue was purified by chromatography on reversed phase (C18) silica gel, eluting first with methanol/0.1% trifluoroacetic acid in water (7:3). This

was followed by silica gel chromatography of the combined fractions, eluting with ethyl acetate/hexane (3:7), to give a colorless oil (183 mg, 50% yield). MS:  $M+H = 568$ .  $^1\text{H-NMR}$ : 7.38 - 7.15 (m, 10H), 6.85 - 6.70 (set of 4 s, 2H), 5.78 - 5.73 (m, 1H), 5.42 - 4.60 (2 sets of m, 1H), 4.07 - 3.67 (m, 10H), 2.97 - 2.85 (m, 1H), 2.72 - 2.53 (m, 2H), 2.42 - 2.08 (m, 3H), 1.85 - 1.00 (m, 5H). LC-MS: System 2 (4 min gradient),  $t_R = 4.6$  min. Anal.  $\text{C}_{32}\text{H}_{35}\text{NO}_6\text{F}_2 \cdot 0.25 \text{CH}_3\text{COOC}_2\text{H}_5$ , C = 67.10, H = 6.56, N = 2.13, F = 6.85 (found), C = 67.22, H = 6.32, N = 2.38, F = 6.44 (calcd.).

## 10 Example 8



The coupling of L-proline with the *p*-(methylthio)phenolic ester of  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetic acid was carried out as described for example 5. The intermediate trimethoxyphenyldifluoroacetamidoproline derivative (0.455 g, 1.26 mmol) was esterified with 3-(3,4,5-trimethoxyphenyl)propan-1-ol as described in example 6 to give the trimethoxyphenylpropyl ester in 42% as a colorless oil. MS:  $M+H = 568$ .  $^1\text{H-NMR}$ : 6.89 (s, 2H), 6.43 (s, 2H), 4.61 (m, 1H), 4.22 (m, 1H), 4.10 (m, 1H), 3.88 (m, 9H), 3.84 (m, 9H), 3.57 (m, 2H), 2.67 (m, 2H), 2.20 (m, 1H), 1.96 (m, 5H). LC-MS: System 2 (8 min gradient),  $t_R = 7.0$  min.

**Example 9**

The synthesis of this compound was carried out as as described for  
5 example 7. N-Boc pipecolic acid (345 mg, 1.5 mmol) was esterified with 3-(3,4,5-trimethoxyphenyl)propan-1-ol to obtain the trimethoxyphenylpropyl ester in 52% yield. MS: M+H = 438. LC-MS: System 2 (4 min gradient),  $t_R$  = 4.2 min. A portion of this Boc-protected ester (325 mg, 0.744 mmol) was deprotected and acylated as described in  
10 example 7 with  $\alpha,\alpha$ -difluoro-3,4,5-trimethoxyphenylacetyl chloride to obtain 183 mg (42%) of the desired compound. MS: M+H = 582.  $^1\text{H-NMR}$ : 6.84 - 6.38 (set of 4 s, 4H), 5.42 - 4.55 (2 x m, 1H), 4.24 - 3.75 (m, 21H), 3.04 - 2.96 (m, 1H), 2.67 - 2.54 (m, 2H), 2.36 - 2.10 (m, 1H), 2.03 - 1.15 (m, 7H). LC-MS: System 2 (4 min gradient),  $t_R$  = 4.0 min. Anal.  $\text{C}_{29}\text{H}_{37}\text{F}_2\text{NO}_9 \cdot 0.25$   
15  $\text{CH}_3\text{COOC}_2\text{H}_5$ , C = 59.66, H = 6.63, N = 2.24, F = 6.70 (found), C = 59.69, H = 6.51, N = 2.32, F = 6.29 (calcd.).

**Example 10****FKBP12 Rotamase Inhibition Assay**

20 The rotamase activity of FKBP-12 was measured by an adaptation of the assay described by Kofron et al. (*Biochemistry*, 30, pp. 6127-6134 (1991)). The assay was carried out at 4°C with 1 mg chymotrypsin/mL of assay solution with succinyl-Ala-Leu-Pro-Phe-p-nitroanilide as the substrate. Chymotrypsin rapidly hydrolyzes the peptide bond on the C-terminal side

of the Phe of the *trans* form of the peptide and releases the chromogenic p-nitroaniline. The rate of the reaction is controlled by the rate of conversion of the *cis* form of the peptide to the *trans*-form, the reaction catalyzed by FKBP12. The apparent  $K_i$  values of compounds of formula I  
5 for inhibition of the rotamase activity were determined by measuring decreases in the first order rate constant of the reaction catalyzed by FKBP12 as a function of the concentrations of the compounds described herein.  $K_i$  is the concentration of the compound that causes 50% inhibition of rotamase activity which is indicative of neurite outgrowth  
10 activity. The results are presented in Table I.

#### Example 11

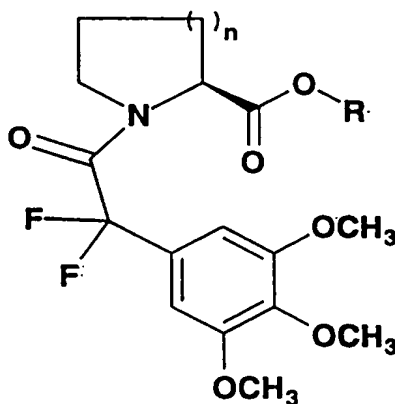
##### Assay of Neurite Outgrowth in PC12 Cell Cultures

PC-12A rat pheochromocytoma cells are maintained in Dulbecco's  
15 modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% calf serum at 37°C and 5% CO<sub>2</sub>. Cells to be assayed are plated at 10<sup>4</sup> per well of a 24 well plate and allowed to attach for 4-18 h. The medium is then replaced with DMEM plus 0.1% BSA (bovine serum albumin), submaximal concentrations of NGF (nerve growth factor) (as  
20 determined by neurite outgrowth assay), and varying concentrations of the FKBP12 binding compound (0.1nM-10μM) in a final concentration of 0.25% DMSO (dimethylsulfoxide). Control cultures are treated with NGF in the absence of the FKBP12 binding compound. After 72 hours, cultures are fixed with 4% formalin in PBS (phosphate buffered saline), stained  
25 with Commassie Blue, and approximately 200 cells are counted in random fields of each well. Cells with neurites longer than one cell diameter are counted as a percentage of total number of cells.

The FKBP12 binding compounds of formula I utilized in this invention cause a significant increase in neurite outgrowth over control cultures.

5        Additionally, compounds of this invention may also show benefit as reversers of multidrug resistance (MDR) in cancer chemotherapy and as agents for the treatment of HIV infection. Nonimmunosuppressive compounds possessing the structural elements of the FKBP12 binding portion of FK506 have shown utility in reversing P-glycoprotein mediated  
10    MDR (U.A. Germann, et al., *Anti-Cancer Drugs*, 8, pp. 125-140 (1997)). In addition, there has been no direct correlation shown between rotamase inhibitory activity and MDR reversing activity (J.R. Hauske, et al., *Bioorg. Med. Chem. Lett.*, 4, pp. 2097-2102 (1994)). In the area of HIV infection, it is known that immunophilins, including the FK506 binding proteins  
15    (FKBPs), are involved in facilitating binding of the HIV envelope protein gp120 to host CD4 receptors (M.M. Endrich, et al., *Eur. J. Biochem.*, 252, pp. 441-446 (1998)), and that FK506 inhibits the growth of HIV-infected cells (A. Karpas, et al., *Proc. Natl. Acad. Sci USA*, 89, pp. 8351-8355 (1992)).

20    Table 1. Rotamase inhibition data with selected examples



Example	n	R	K <sub>i</sub> (nM)	Percent Inhibition at 10 $\mu$ M
1	1	3-phenylpropyl	1300	97
2	1	3-(3-pyridyl)propyl	877	97
3	1	4-[7-(3-pyridyl)-1-phenylheptyl]	104	97
4	2	4-[7-(3-pyridyl)-1-phenylheptyl]	19	98
5	1	3-(1-phenyl-3-methylbutyl)		70
6	1	1-(1,3-diphenylpropyl)	83	100
7	2	1-(1,3-diphenylpropyl)		94
8	1	3-(3',4',5'-trimethoxyphenyl)propyl		38
9	2	3-(3',4',5'-trimethoxyphenyl)propyl	109	99

If pharmaceutically acceptable salts of the compounds of formula I are used, those salts are preferably derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, aspartate, bisulfate, butyrate, citrate, fumarate, hydrochloride, hydrobromide, hydroiodide, lactate, maleate, oxalate, persulfate, propionate, succinate, tartrate. Base salts include ammonium salts, alkali metal salts, such as sodium and potassium salts, alkaline earth metal salts, such as calcium and magnesium salts, salts with organic bases, such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth.

Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such

as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium  
5 chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The compositions of the present invention may be administered  
10 orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir.

It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body  
15 weight, general health, sex, diet, time of administration, rate of excretion, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of compound of formula I will also depend upon the particular FKBP12 binding compound in the composition.

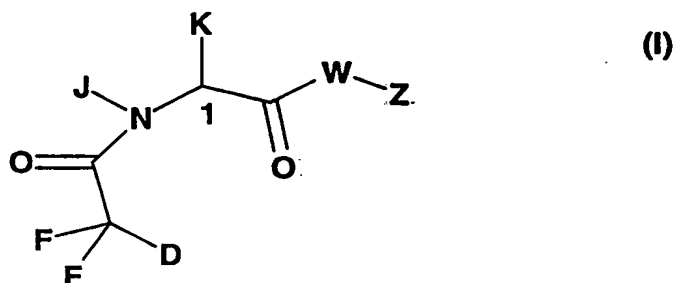
20 The amount of compound of formula I utilized in these methods is between about 0.01 and 100 mg/kg body weight/day.

CLAIMS

What is claimed is:

1. A compound having the formula (I):

5



and pharmaceutically acceptable salts thereof:

- 10 wherein W is CH<sub>2</sub>, O, NH, or N-(C<sub>1</sub>-C<sub>4</sub>)-alkyl;

wherein J is hydrogen, (C<sub>1</sub>-C<sub>4</sub>)-alkyl or benzyl;

- wherein K is (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, benzyl or  
 15 cyclohexylmethyl, or wherein J and K may be taken together to form a 5-7  
 membered heterocyclic ring which may contain a heteroatom selected  
 from the group consisting of O, S, SO, and SO<sub>2</sub>;

wherein the stereochemistry at carbon position 1 is R or S;

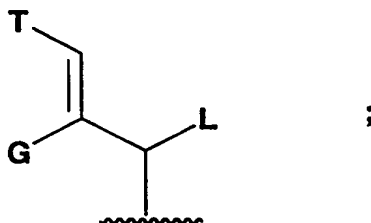
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wherein Z is Q or -(CH<sub>2</sub>)<sub>m</sub> -C(H)Q'A;

wherein m is 0-3;



wherein Q is hydrogen, CHL-Ar, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl, Ar substituted (C<sub>1</sub>-C<sub>6</sub>)-alkyl, (C<sub>2</sub>-C<sub>6</sub>)-alkenyl or



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wherein L and G are independently hydrogen, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl;

- 10 wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C<sub>1</sub>-C<sub>4</sub>)-alkyl or O-(C<sub>2</sub>-C<sub>4</sub>)-alkenyl and carbonyl;

- wherein D is (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or  
 15 branched alkenyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl or (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl substituted with (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>4</sub>)-straight or branched alkenyl, O-(C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, O-(C<sub>2</sub>-C<sub>4</sub>)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C<sub>1</sub>-C<sub>4</sub>)-alkyl or (C<sub>2</sub>-C<sub>4</sub>)-alkenyl]-Ar or Ar;

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- wherein Ar is a carbocyclic aromatic group selected from the group consisting of phenyl, 1-naphthyl, 2-naphthyl, indenyl, azulenyl, fluorenyl, and anthracenyl; or a heterocyclic aromatic group selected from the group consisting of 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-  
 25 pyridyl, 4-pyridyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl,

isoxazolyl, isothiazolyl, 1,2,3-oxadiazolyl, 1,2,3-triazolyl, 1,3,4-thiadiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, 1,3,5-triazinyl, 1,3,5-trithianyl, indolizinyl, indolyl, isoindolyl, 3H-indolyl, indolinyl, benzo[b]furanyl, benzo[b]thiophenyl, 1H-indazolyl, benzimidazolyl, benzthiazolyl, purinyl, 5 4H-quinolizinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 1,8-naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, and phenoxazinyl;

wherein Ar may contain one to three substituents which are  
10 independently selected from the group consisting of hydrogen, halogen, hydroxyl, hydroxymethyl, nitro, trifluoromethyl, trifluoromethoxy, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl, O-[(C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl], O-benzyl, O-phenyl, 1,2-methylenedioxy, amino, carboxyl, N-[(C<sub>1</sub>-C<sub>5</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>5</sub>)-straight or  
15 branched alkenyl] carboxamides, N,N-di-[(C<sub>1</sub>-C<sub>5</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>5</sub>)-straight or branched alkenyl] carboxamides, N-morpholinecarboxamide, N-benzylcarboxamide, N-thiomorpholinocarboxamide, N-picolinoylcarboxamide, O-X, CH<sub>2</sub>-(CH<sub>2</sub>)<sub>p</sub>-X, O-(CH<sub>2</sub>)<sub>p</sub>-X, (CH<sub>2</sub>)<sub>p</sub>-O-X, and CH=CH-X;

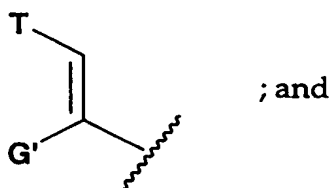
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wherein X is 4-methoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrazyl, quinolyl, 3,5-dimethylisoxazolyl, isoxazolyl, 2-methylthiazoyl, thiazoyl, 2-thienyl, 3-thienyl, or pyrimidyl;

25 wherein p is 0-2;

wherein Q' and A are independently hydrogen, Ar, (C<sub>1</sub>-C<sub>10</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>10</sub>)-straight or branched alkenyl or alkynyl,

- (C<sub>5</sub>-C<sub>7</sub>)cycloalkyl substituted-straight (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl substituted (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl, or Ar substituted (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl,
- 5 (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl wherein, in each case, any one of the CH<sub>2</sub> groups of said alkyl, alkenyl or alkynyl chains may be optionally replaced by a heteroatom selected from the group consisting of O, S, SO, SO<sub>2</sub> and NR, wherein R is selected from the group consisting of hydrogen, (C<sub>1</sub>-C<sub>4</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>4</sub>)-straight or branched
- 10 alkenyl or alkynyl, and (C<sub>1</sub>-C<sub>4</sub>)-bridging alkyl wherein a bridge is formed between the nitrogen and a carbon atom of said heteroatom-containing chain to form a ring, and wherein said ring is optionally fused to an Ar group; or



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wherein G' is hydrogen, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl or (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl.

- 20 2. A compound of claim 1 wherein Z is  $-(CH_2)_m-C(H)Q'A$ .

3. A compound of claim 2 wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is 3-phenylpropyl; and A is 3-(3-pyridyl)propyl.

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4. A compound of claim 2 wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is 3-phenylpropyl; and A is 3-(3-pyridyl)propyl.

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5. A compound of claim 2 wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is phenyl; and A is 2-phenylethyl.

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6. A compound of claim 2 wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is phenyl; and A is 2-phenylethyl.

20

7. A compound of claim 2 wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; and Q' and A are both (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyls substituted at the terminal end with a

25 (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.

8. A compound of claim 2 wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; and Q' and A are both (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyls substituted at the terminal end with a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.
9. A compound of claim 2 wherein J and K are taken together to form a piperidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar; and A is a (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyl substituted at the terminal end with a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.
10. A compound of claim 2 wherein J and K are taken together to form a pyrrolidine ring; the stereochemistry at carbon 1 is S; W is oxygen; m is 0; D is 3,4,5-trimethoxyphenyl; Q' is a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar; and A is a (C<sub>1</sub>-C<sub>4</sub>)-straight chain alkyl substituted at the terminal end with a (C<sub>5</sub>-C<sub>7</sub>)-cycloalkyl, (C<sub>5</sub>-C<sub>7</sub>)-cycloalkenyl or Ar.
11. A pharmaceutical composition which comprises as an active ingredient an amount of a compound as claimed in any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, effective for stimulating neurite growth in nerve cells, and one or more pharmaceutically acceptable carriers, excipients or diluents thereof.
12. A method for stimulating neurite growth in nerve cells comprising the step of contacting said nerve cells with a composition comprising a neurotrophic amount of a compound with affinity for an FK-506 binding protein as claimed in any one of claims 1-10.

13. A method for stimulating neurite growth in nerve cells comprising the step of contacting said nerve cells with a composition comprising a neurotrophic amount of a compound with affinity for FKBP12 as claimed in any one of claims 1-10.

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## INTERNATIONAL SEARCH REPORT

Application No.  
PCT/US99/11348**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A61K 31/34, 31/44; C07D 211/68, 207/06

US CL :514/279, 468; 546/194; 548/579

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/279, 468; 546/194; 548/579

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EAST AND WEST DATABASES

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,330,993 A (ARMISTEAD ET AL.) 19 JULY 1994, COL. 4, LINES 4-68.	1, 6, 10
A	US 5,192,773 A (ARMISTEAD ET AL.) 09 MARCH 1993, COL. 3, LINES 14-68.	1, 6, 10

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

07 OCTOBER 1999

Date of mailing of the international search report

29 OCT 1999

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